OIST PREreview JC - "Disentangling unspecific and specific transgenerational immune priming components in host-parasite interactions"

Maggi Brisbin[1], Yuka Suzuki[1], Julian K[1], and Jigyasa Arora[1]

[1]Okinawa Institute of Science and Technology Graduate School

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Disentangling unspecific and specific transgenerational immune priming components in host-parasite interactions

Frida Ben-Ami, Christian Orlic, Roland R. Regoes

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Overview and take-home messages:

In this study, the authors tackle the topic of transgenerational immune priming in invertebrates. The authors designed a large experiment taking advantage of clonal *Daphnia* to test whether infecting parental generations with different parasite strains improves the offspring's resistance to that parasite overall and if yes, if they resist that specific strain more effectively than other strains. This experiment essentially tests the specificity of immune priming at a very fine "strain" scale. The results did not support parental infection strain differentially affecting offspring resistance to different strains, suggesting that immune priming is not specific to the strain level in this system. However, a mathematical model the authors developed for that study fits the data exceptionally well, which means this model could potentially be used in a predictive manner for this or similar systems. Additionally, the unexpected result that one strain actually facilitates specific infection in the offspring is surprising and opens the door to additional inquiry and future experimentation. Overall this study is very interesting and well-presented, but there are a few concerns that could be addressed and improved in the next version of the manuscript.

Positive feedback:

- The *Daphnia-Pasteuria* system is an interesting and appropriate system to study transgenerational effects. The fact that Daphnia is parthenogenic and produces clonal offspring is an amazing advantage that the authors utilize well in this study.
- Transmitting acquired traits, like immune memory, from parents to offspring is an exciting prospect. However, in this direct, single-generation transmission, there are many confounding factors. Parental infection could affect offspring in myriad ways, such as decreased offspring size to name only one. This is especially true in this system where the parasite directly influences host reproduction. It would be extremely interesting to see the authors extend this work into the F2 and F3 generations and determine if immune priming persists past the generation directly derived from the infected parent.

- The model applied in this study fits the data very well and is not the type of model typically considered by microbial ecologists. Other species interactions and specificity studies in different systems could benefit greatly by exploring the use of frailty mixed models as were adapted for this paper.

Major concerns:

- The experimental design is very impressive, considering the large number of comparisons included in the experiment. A major concern for us was the seeming lack of biological replicates, or at least an obvious description of them. The methods section indicates that each infection experiment was carried out in a 100 mL jar, but it seems like there was one jar per treatment combination (it is possible we are missing something in our reading). We feel it is important to clearly state how many jars were prepared per treatment and if it was only one jar per treatment to discuss why this was the case. We understand that this is large experiment and it was likely very time-consuming to maintain all of the treatments, which could have made replication simply not feasible. However, biological replication would greatly increase the reliability of the results and it is important to recognize and discuss this. This comes especially into play when the authors discuss the variability in results between the current experiment and a previously published experiment. If there is biological replication in both experiments, readers can better evaluate whether the observed variation was within a normal range of variation for this system. Likewise, error bars in Figure 2 would make the results easier to evaluate. If we have interpreted the text properly and biological replication was not included, the authors could consider explaining why this was the case and explicitly how they account for it in the results.
- In the introduction, methods, and results section the authors refer to parasite "strains," but switch to parasite "isolates" in the discussion where it becomes clear that this distinction is rather important to interpreting the results of the study. When they are referred to as strains, we assumed that each strain was a genetically distinct and genetically homogenous culture. In the discussion, there is a more comprehensive description of the "strains" and they were more accurately described as "isolates." Although the isolates are more representative of natural infections, their very nature makes the experiments very difficult to replicate. It would be helpful to use more controlled isolates (known strain composition) and do 16S monitoring to determine if strain or isolate composition itself evolves and changes over time .

Minor concerns:

- Figure 5 is a very clear and informative figure. As readers, we can easily observe that the ID50 decreases two-fold in primed offspring compared to the control. In addition, the anomalous increase in P5 infection is also straightforward. It is more difficult to see these trends in Figure 2. The authors might consider including ID50 in this plot as well or to remove it completely.
- Although it is very clear that the ID50 changes between treatments, it is difficult for us to put the change in context without more discussion. Is it a big change? How does the change compare to studies in other model organisms?
- We recognize that the model used in this study is a central strong point of the work. However, since we were not previously familiar with frailty models, it would be helpful to have more discussion about why this model was chosen and which alternatives were considered.
- Since this work focused on disentangling specific and unspecific priming, it would be really nice to see the relative significance of specific and unspecific effects, especially in a figure.
- The authors mention the applicability of this study to the experimental assessment of vaccines, but the connection feels a little tenuous because vaccines are generally applied to vertebrates and vertebrate and invertebrates have very different immune responses. The authors may consider discussing these differences in more detail and providing a few concrete examples of how this study relates to vaccine assessment.
- The authors state that this study is not concerned with the molecular methods involved in immune priming, but it may still be beneficial to discuss molecular mechanisms in relation to the results found here. Perhaps the mechanisms for immune priming in invertebrates are not specific enough to

differentiate between strains and would therefore support the results found in this study.
- The authors refer to Ben-Ami et al., 2008, 2010 as "we". Please consider that this might not be common practice.
- It would be helpful if the authors discuss future directions for this work. We are really interested to see where they feel this work should go next!

We thoroughly enjoyed reading and discussing this preprint in our journal club and thank the authors for posting their work on the bioRxiv. We sincerely hope that our comments are helpful and we look forward to seeing the final published version!

Best wishes,

The OIST Ecology and Evolution Preprint Journal Club